FRAGRANCE MATERIALS ASSOCIATION OF THE UNITED STATES
Suite 925, 1620 I Street, N.W.
Washington, D.C. 20006
(202) 293-5800
Facsimile (202) 463-8998

Dr. Scott Masten
NIEHS/NTP
P.O. Box 12233
Research Triangle Park, North Carolina 27709

2 February 2001

RE: National Toxicology Program; Request for Comments on Substances Nominated to the National Toxicology Program (NTP) for Toxicological Studies. 65 Fed. Reg. 75727 (4 December 2000)

Dear Dr. Masten:

On behalf of the members of the Fragrance Materials Association of the United States (FMA), we provide these comments on the nomination of lemon oil and lime oil for inclusion in the testing program administered by the National Toxicology Program (NTP). 65 Fed. Reg. 75727 (4 December 2000). FMA believes that the inclusion of lemon oil and lime oil in the program is inconsistent with the criteria for the nomination and selection of substances for testing by NTP.

FMA is the national association of the manufacturers of fragrances. FMA's members produce the vast majority of fragrances that are incorporated into a wide variety of consumer products. FMA members have extensive experience with lemon oil and lime oil, especially with respect to the composition of these oils. These natural oils have been used for hundreds of years in myriad foods, beverages, cosmetics, and consumer products.

The Nomination and Selection of Substances for Testing by NTP

The process by which substances are nominated and selected for testing by NTP is summarized in the current proposal, and in a previous proposal published in March 2000. 65 Fed. Reg. 11329 (2 March 2000). In this proposal, NTP stated,

> The NTP actively seeks to identify and select for study chemicals and agents with the highest potential for adversely impacting public health. . . . substances selected generally fall into two broad overlapping categories: (1) Those substances of greatest concern for public or occupational health based on the extent of human exposure and suspicion of toxicity, and (2) substances for which toxicological gaps exist and additional studies would aid in assessing potential human health risks by facilitating cross-species extrapolation and evaluation of dose-response relationships. 65 Fed. Reg. at 75727.

As can be readily seen from the "background summary" for the nomination, "Lemon Oil, Lime Oil" (dated October 2000 and available on the NTP website), the available data on lemon oil and lime oil do not indicate that they are substances with great concern for public health, nor are they substances for which significant "toxicological gaps" exist. Also, these oils have extremely low levels of human exposure further alleviating concern about public health effects.

The Basis for the Nomination

Lemon oil and lime oil were nominated for testing in the NTP program by the Center for Food Safety and Applied Nutrition of the Food and Drug Administration (FDA CFSAN). The background summary for the nomination states in Section 1.0,

> Lemon oil and lime oil were selected by (CFSAN) for photogenotoxicity and photocarcinogenesis studies since these phototoxic oils contain furocoumarins. Furocoumarins such as 5-methoxypsoralen have been shown to be photocarcinogenic and are present in lemon and lime oils.

It is important to note that the background summary report does not include a summary of the abundant literature on the safety testing of lemon oil and lime oil, nor does it contain comments on its very long history of safe use in many products, including cosmetics that are applied to the skin. A review of this information leads to the conclusion that lemon oil and lime oil are not "agents with the highest potential for adversely impacting public health," a key criterion for determining appropriate candidates for NTP testing.

Current Uses of Lemon Oil and Lime Oil Do Not Result in Significant Exposure to Naturally Occurring Psoralens

The global trade in lemon oil and lime oil is subject to an effective program of industry self-regulation administered by the International Fragrance Association (IFRA) based in Brussels. FMA is a member of IFRA, and as a condition of membership in FMA, FMA's members must agree to abide by the guidelines administered by IFRA.

IFRA guidelines indicate that lemon oil cold pressed should not be used such that the level in the consumer product exceeds 2%. The level in lime oil cold pressed should not exceed 0.7%. The policy of industry-wide self-regulation of these oils is based on the phototoxic potential of the psoralens contained in cold-pressed oils in the marketplace. The guidelines are intended to maintain extremely low levels of psoralens in cosmetic products.

Section 5.0 of the background summary report states that lemon oil and lime oil are found in only 98 and 14 cosmetic formulations, respectively, in the U.S. according to FDA's Voluntary Cosmetics Registration Program. It is highly unlikely that such a small number of products would result in significant exposure in consumers to psoralens naturally occurring in lemon oil and lime oil.

The Nomination of Lemon Oil and Lime Oil is Premature Based on an Analysis of the Available Data

A basis for the nomination of lemon oil and lime oil is that high concentrations of these dermally-applied oils are phototoxic, and that their phototoxicity has been strongly associated with the presence of the naturally occurring psoralens, 5-methoxypsoralen and oxypeucedanin. Although the short-term phototoxicity of these two psoralens has been adequately characterized, little information is available on their chronic toxicity. According to the background summary, the photogenotoxic potential of 5-methoxypsoralen is known but the photogenotoxic potential of oxypeucedanin is unknown, hence the request to investigate the photogenotoxic potential of oxypeucedanin.

FMA is concerned that the nomination of lemon oil and lime oil is premature. The investigation of the photogenotoxic potential of oxypeucedanin and other related psoralens is the subject of the initial study. We are of the opinion that it is first necessary to properly characterize the photogenotoxic potential of the chemically-identified psoralens in a series of validated assays.

Currently, the field of *in vitro* photogenotoxicity testing abounds with many studies in which the experimental variables (conditions for photoactivation, effects of photoactivation on cell cytotoxicity, etc.) have not been adequately standardized.

It is incumbent on NTP to validate selected photogenotoxicity assays prior to investigating lemon oil and lime oil, or naturally-occurring psoralens in these oils. As the NTP is well aware, the extrapolation of the results of validated *in vitro* assays in prokaryotic and isolated eukaryotic cell systems

to the potential for genotoxicity *in vivo* is difficult, to say the least. Therefore it is also incumbent on the NTP to develop an *in vivo* model in an appropriate species to assure that the results of *in vitro* assays can be used to assess the relevance of photogenotoxicity assays.

If Testing Proceeds, the Oils and Other Substances Tested Must Reflect Real Human Exposure

In the event that NTP decides to study lemon oil and lime oil *per se*, FMA requests that they be consulted on the types of lemon oil and lime oil that are used in assessing photogenotoxicity. Use of oils of high psoralen content from selected geographical regions and produced under certain growing conditions are not appropriate for testing given their limited use in the marketplace. As described above, IFRA Guidelines significantly limit human exposure to psoralens in lemon oil and lime oil from cosmetic products. Any testing of these oils should reflect this reality.

We also emphasize that results of experiments with oxypeucedanin ("neat" or dissolved in alcohol), or natural oils applied neat to the skin, are not relevant to real human exposure. No one applies these oils directly to the skin, they are applied diluted in a cosmetic base that exhibits different absorption dynamics than if the substances were applied neat. Furthermore, application of lemon oil or lime oil in a cosmetic base significantly alters the exposure of psoralens to sunlight. Therefore, the interpretation of the results of any *in vitro* or *in vivo* experiments must account for this difference in the mode of exposure.

Conclusions

The available toxicology and exposure information demonstrate that lemon oil and lime oil do not meet the criteria for inclusion in the NTP testing program. We respectfully request that lemon oil and lime oil be removed from consideration, and that the resources that would have been assigned to testing these oils be re-assigned to substances that meet the NTP's criteria for inclusion in their testing program.

If NTP decides to proceed with the testing of lemon oil and lime oil, we request that NTP fully describe the protocols to be employed in the testing, and provide an opportunity for public review of the protocols. We also request the opportunity to provide our comments on the nature of the oils to be tested so that we may assist NTP in selecting oils that reflect the reality of consumer exposure.

Thank you for your consideration of our comments.

Sincerely,

Glenn Roberts
Executive Director